

**UNITED STATES AIR FORCE
ARMSTRONG LABORATORY**

**Determination of Partition Coefficients
for Trichloroethanol (TCOH) and
Chloral Hydrate (CH)-Two Metabolites
of Trichloroethylene (TCE)**

C. Seckel

ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

**J.R. Creech
R.K. Black
B.L. Garrity**

Toxicology Division
Wright-Patterson AFB, OH 45433-7400

February 1995

19990830 026

Approved for public release; distribution is unlimited.

DTIC QUALITY INSPECTED 4

**Occupational and Environmental Health
Directorate
Toxicology Division
2856 G Street
Wright-Patterson Air Force Base, OH
45433-7400**

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Armstrong Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Center
Cameron Station
Alexandria, Virginia 22314

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

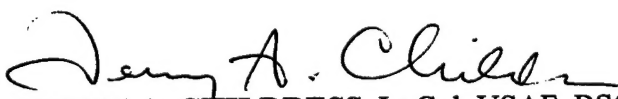
AL/OE-TR-1995-0023

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER


TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave Blank)		2. REPORT DATE February 1995		3. REPORT TYPE AND DATES COVERED Interim -August 1994 - January 1995
4. TITLE AND SUBTITLE Determination of Partition Coefficients for Trichloroethanol (TCOH) and Chloral Hydrate (CH) - Two Metabolites of Trichloroethylene (TCE)			5. FUNDING NUMBERS Contract F33615-90-C-0532 PE 63716D PR 4223 TA 4223OT WU 4223OT01	
6. AUTHOR(S) C. Seckel, J.R. Creech, R.K. Black, and B.L. Garrity				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) ManTech Environmental Technology, Inc. P.O. Box 31009 Dayton, OH 45437-0009			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Armstrong Laboratory, Occupational and Environmental Health Directorate Toxicology Division, Human Systems Center Air Force Materiel Command Wright-Patterson AFB OH 45433-7400			10. SPONSORING/MONITORING AGENCY REPORT NUMBER AL/OE-TR-1995-0023	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Trichloroethylene (TCE), a common groundwater contaminant, has been shown to be carcinogenic in some animal species. To determine the risk in humans, the use of physiologically-based pharmacokinetic models (PBPK) has become increasingly the method of choice. Formerly it was a common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence, it is now recognized that the dose delivered to the target organ is more accurate. Determination of partition coefficients (the solubility of the chemical in tissue) is one element important in the development of the PBPK model. This study focuses on two metabolites of TCE, chloral hydrate (CH) and trichloroethanol (TCOH). All studies were conducted in B ₆ C ₃ F ₁ mice since this species was used in the NCI/NTP carcinogenicity studies on TCE. Partition coefficients for CH and TCOH were determined using the nonvolatile method of Jepson <i>et al.</i> , (Fund. and Appl. Tox 22: 1994). Tissues used in CH studies were pre-treated with 20% lead acetate to insure no metabolism occurred during the incubation period.				
14. SUBJECT TERMS Trichloroethylene Trichloroethanol PBPK Partition coefficients Chloral hydrate Metabolism B ₆ C ₃ F ₁			15. NUMBER OF PAGES 18	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

THIS PAGE INTENTIONALLY LEFT BLANK

Preface

This is one of a series of technical reports to describe results of experimental laboratory work conducted at the Toxicology Division , Armstrong Laboratory Wright-Patterson AFB in support of a project to construct a physiologically-based pharmacokinetic model (PBPK) for trichloroethylene in B6C3F1 mice. This interim report focuses on the partition coefficients using the non-volatile method for two major metabolites, Trichloethanol (TCOH) and Chloral Hydrate(CH). The report covers the period from August 1994 to January 1995.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on the Care and Uses of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, Department of Health and Human Services, National Institute Of Health Publication 385-23, 1986 and the Animal Welfare Act of 1966, as amended.

Table of Contents

Section	Page
I. Introduction	4
II. Physiologically-based Pharmacokinetic Models	6
a. Development	
b. utilization	
III. Methods	7
a. chemicals	
b. animals	
c. partition coefficients	
IV. Results	8
V. Discussion	9
VI. References	9

List of Figures

I. Metabolic Pathway for Trichloroethylene	5
--	---

List of Tables

I. Partition Coefficients	8
a. Chloral hydrate	
b. Trichloroethanol	

Abbreviations

TCOH	Trichloroethanol
CH	Chloral Hydrate
TCE	Trichloroethylene
PBPK	Physiologically-based pharmacokinetic
TCA	Trichloroacetic acid
DCA	Dichloroacetic acid
EPA	Environmental Protection Agency
NCI	National Cancer Institute
NTP	National Toxicology Program
ml	milliliter
°C	degree centigrade
rpm	revolutions per minute
NMWL	nominal molecular weight limit
psi	pounds per square inch
mm	millimeter
M	meter
µg	microgram

INTRODUCTION

Trichloroethylene (TCE) is a solvent that has been used for degreasing of metals. It has also been used for other purposes: a solvent in adhesives, paint stripping, dry cleaning, an anesthetic, and disinfectant. Because of this widespread use of TCE, it has become a common environmental contaminant. It was one of the 10 most commonly detected chemicals at hazardous waste site (1). Exposure of humans to TCE in a variety of settings is of concern primarily because of its carcinogenic potential. TCE has been shown to cause cancer in mice and rats (2-6).

From these studies, it appears that the metabolites of TCE rather than the parent compound are responsible for cytotoxicity and carcinogenicity in the liver and other organs. It has been known that TCE is extensively metabolized in the body to trichloroethanol (TCOH), TCOH-glucuronide, and trichloroacetic acid (TCA). Dichloroacetic acid (DCA) which is also formed and TCA are known inducers of hepatic tumors in mice (7-10). The 1985 EPA health assessment document (11) on TCE noted that the metabolic pathways for TCE appear to be qualitatively similar in mice, rats and humans. The metabolism is quantitatively different with metabolic capacity lower in rats than in mice.

Butler (12) found that chloral hydrate, a hypnotic widely used as an anesthetic and as a drug, was also metabolized to TCOH, TCOH-glucuronide, and TCA. This lead to the hypothesis that chloral hydrate was an intermediate metabolite of TCE. He could not, however, demonstrate the presence of chloral hydrate in plasma and urine. Chloral hydrate has since been found in the plasma and microsomes from rats and humans (13-19).

Thus, the metabolic pathways of TCE (Fig.1) have been studied extensively and reexamined.

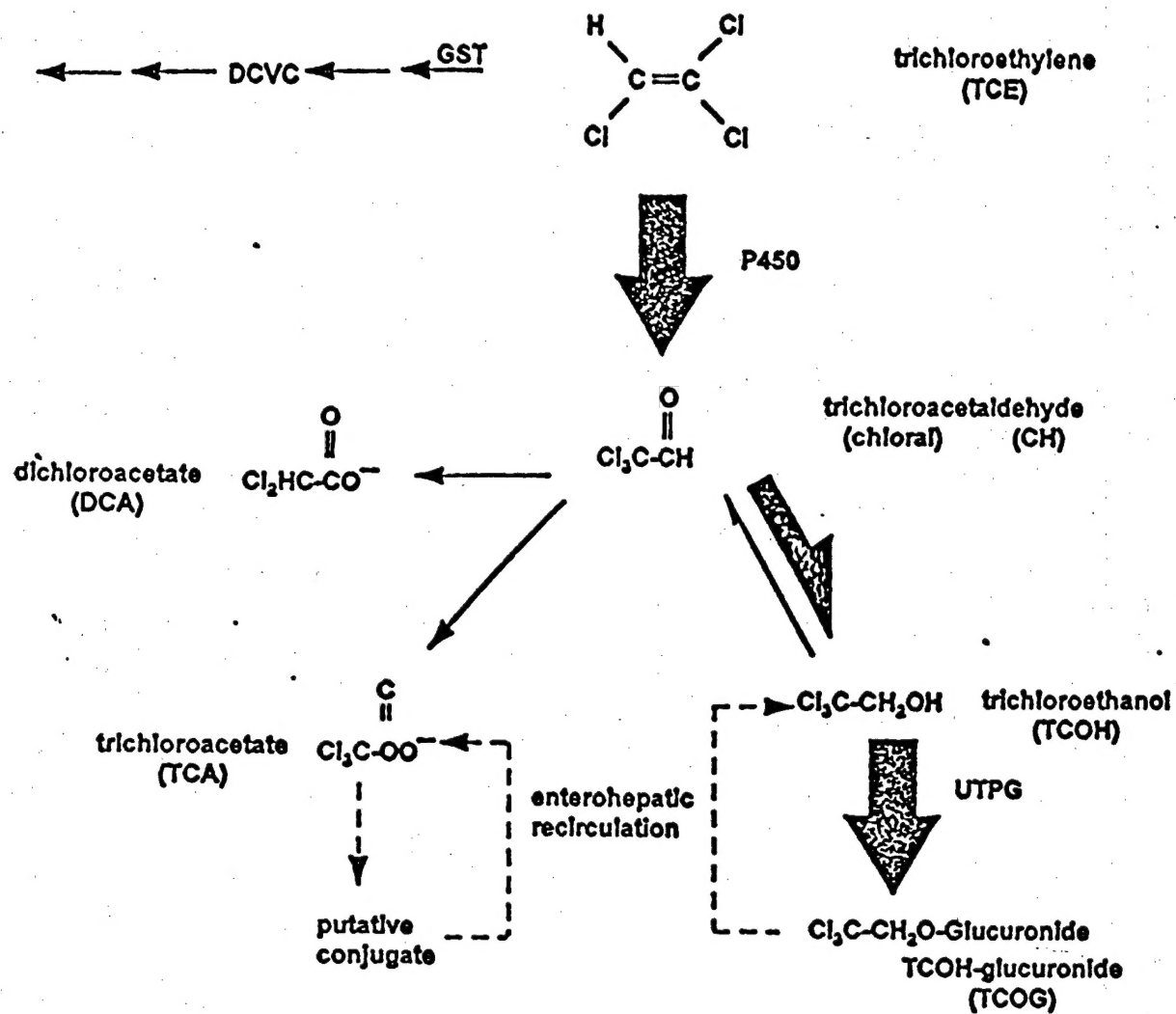


Figure 1. Selected steps in the Metabolism of Trichloroethylene

Development of PBPK models and their utilization

To aid in the understanding of the disposition of a chemical within the body, scientists have developed the physiologically-based pharmacokinetic model (PBPK). Such models support the four important extrapolations: high-low dose, dose route, interspecies and altered exposure patterns. Formerly it was a common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence, it is now recognized that the dose delivered to the target organ is more accurate.

A number of PBPK Models for TCE have been developed. Sato et al. (19) focused on respiratory exposure for humans to TCE. It was a 3 compartmental model, with intercompartmental exchange of TCE governed solely by intertissue diffusion. Metabolic and respiratory excretion was assumed to occur in the richly perfused tissue compartment. Fernandez et al. (20) predicted respiratory elimination of TCE and urinary excretion of TCE metabolites in humans. This model was built on Sato's model with a liver compartment with blood-flow -limited delivery plus a lung compartment for respiratory absorption and elimination. The Ramsey and Andersen model (21) was used by Andersen et al. (22) to predict influence of competitive inhibition on uptake of inhaled TCE in rats. Fisher et al. (23) used the model developed by Andersen and added compartments (mammary tissue, placenta and fetus) to allow for the physiological changes of pregnancy. This model was again modified to account for lactation and nursing (24). The Ramsey and Andersen model with a lung compartment was used by Dallas et al. (25).

Missing, however, from the literature was a PBPK model of TCE in mice. There appears to be a need to develop such a model because many of the positive carcinogenesis studies were carried out in this species.

PBPK models consist of groups of organs (compartments) arranged in anatomical configuration connected by the cardiovascular system. When building the model, 3 groups of parameters are required: (1) organs and tissue volumes, blood flow rates; (2) thermodynamic properties such as partition coefficients; and (3) metabolic constants as well as absorption and excretion. Information about the first group of parameters is available from a variety of literature sources. Partition coefficients or distribution coefficients as a measure of solubility of the chemical in tissue are typically determined experimentally. They represent the ratio of chemical concentration in two interfacing phases when chemical is in equilibrium between 2 phases. For the purposes of PBPK model, the biological phases in which the chemicals are distributed are air, blood, fat, muscle, liver, other tissues and biological fluids. Intravenous dosing and other routes of administration provide the descriptions for parameter 3.

This report focuses of the determination of the partition coefficients for chloral hydrate (CH) and trichloroethanol (TCOH).

Construction of 2 separate models but interlinked for these metabolites that can be used separately or linked with other PBPK models may aid in better understanding the kinetics of parent TRI.

METHODS

Chemicals used: Chloral Hydrate (CAS #302-17-8) and Trichloroethanol (CAS #115-20-8) were obtained from Sigma Chemical. Lead Acetate which was added to inhibit metabolism was obtained from Mallinckrodt. Physiological saline (0.9%) was used as the diluent.

Animals used: B6C3F1 mice used in this study were handled in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health, Publication No. 86-23, 1985; and the Animal Welfare Act of 1966, as amended.

Partition coefficients: The method for nonvolatile chemicals developed by Jepson et al. (26). was used. In this method for each run, (3) B6C3F1 mice were sacrificed using CO₂ and tissues harvested and pooled. Partitions for blood, liver, muscle and fat were determined for trichloroethanol and chloral hydrate. Brain was also included in the partitions for chloral hydrate. Tissues and blood were weighed into 3-20 ml scintillation vials capped with teflon/rubber septa to prevent the absorption of the chemical. The appropriate concentration of chemical in 0.9% saline was added to each vial. Typically, 0.5 gram of blood or tissue and 5 mls of chemical in saline were added to each vial. To prevent possible metabolism of chloral hydrate especially in the blood and the liver, 20% lead acetate was added to each vial. Vials were vortexed at medium speed for 18 hours at 37°C. Supernatant was centrifuged at room temperature for 10 minutes at 1500 rpm. The resulting supernatant was filtered through a prewashed Millipore Ultra-PF low-binding cellulose 10,000 NMWL filter. All millipore cells were rinsed with de-ionized water by applying 40 psi of compressed nitrogen. A syringe with plastic tubing was attached to the bottom opening of each cell so that any excess saline was removed. All cells were used within 2 hours of washing to insure that they did not dry out. The supernatant was filtered through each cell by applying 40 psi of pressure and was kept stirring over a magnetic stirrer to prevent clogging of the filter. Triplicate samples of each filtrate were set up for extraction of chemical. Ethyl acetate was the solvent of choice. Samples were vortexed at medium speed for 30 minutes at 37°C and were centrifuged. The ethyl acetate phase was removed and was analyzed by Hewlett Packard 5890 Series II gas chromatography

equipped with a Hewlett Packard 7673A Autosampler. The amount of ethyl acetate added was dependent on the initial concentration of the chemical so each was in the range of the standard curve. Vials, containing the appropriate chemical concentration; but no tissue, served as a reference or blanks. A PE Nelson Turbochrom v.4.03 Analytical System was used to collect and process data. The GC conditions were: Column - Vocol 30 M X 0.53mm, oven temperature 120°C, electron capture detector temperature 300°C, injector temperature 175°C.

RESULTS

The partition coefficients for CH and TCOH determined in this study are listed in Table 1

Tissue : Saline Partition Coefficients

TISSUE	CHLORAL HYDRATE	
	50µg/ml	100µg/ml
BLOOD	1.86 (0.29)	1.82 (0.54)
FAT	0.68 (0.34)	0.88 (0.38)
LIVER	2.55 (0.43)	2.67 (0.29)
MUSCLE	2.28 (0.47)	2.45 (0.47)
BRAIN	2.48 (0.43)	2.53 (0.92)
LUNG	3.07 (1.31)	2.94 (1.64)

TISSUE	TRICHLOROETHANOL	
	50µg/ml	100µg/ml
BLOOD	2.98 (0.25)	3.48 (1.26)
FAT	5.51 (0.64)	5.31 (0.47)
LIVER	3.81 (0.60)	3.68 (1.37)
MUSCLE	3.33 (1.23)	3.86 (1.57)

partitions listed are the mean with coefficient of variation in parenthesis

DISCUSSION

Because of its environmental and toxicological concerns, there is interest in studying of Trichloroethylene and its metabolites. It is known to cause cancer in various animal species; but its relationship and incidence of causing cancer in humans is not clear. From the carcinogenicity studies, it appears that the metabolites of TCE rather than the parent compound are responsible for cytotoxicity and carcinogenicity in the liver and other organs. Thus, we focused on 2 main metabolites chloral hydrate and trichloroethanol. In the experiments, mouse tissue was used because of the incidence of carcinogenicity in this species.

The use of physiologically-based pharmacokinetic modeling has become increasingly common in risk assessment because of it is amenable to interspecies extrapolation necessary to calculate human tissue dose. However, to have a workable model, one must have dependable data. One set of data is the partition coefficients or distribution coefficients. A typical method to determine the partitions is the vial equilibration method in which a sample of headspace chemical is analyzed. Because of the low volatility of these 2 metabolites, this method was not practical. The nonvolatile method developed by Jepson et al. (26) had been shown to be an adequate alternative method. While partitions are only part of the model, these numbers combined with other information included in the PBPK model can help make conclusions about risk.

REFERENCES

1. Josephson J. Implementing superfund. Environ. Sci. Technol., 20,23 1986.
2. National Toxicology Program, Carcinogenesis Bioassay of Trichloroethylene (without epichlorohydrin) in F344/N rats and in B6C3F1 mice (gavage studies). US Department of Health and Human Services Tech Rep No. 243, NIH Publ. No. 83-1799, 1983.
3. Fukuda, K., Takemoto, K., and Tsuruta, H., Inhalation carcinogenicity of trichloroethylene in mice and rats. Ind. Health. 21:243, 1983.
4. National Cancer Institute, Carcinogenesis Bioassay of Trichloroethylene. CAS No.79-01-6, NCI-CG-TR-2, Washington, DC 1983.
5. Maltoni, C., Lefemine, G., Cotti, G. and Perino, G. Long-term

carcinogenicity bioassays on trichloroethylene administered by inhalation to Sprague-Dawley rats and Swiss and B6C3F1 mice. Ann. N.Y. Acad. Sci. 534, 316 1988.

6. National Toxicology Program, Toxicology and Carcinogenesis studies of Trichloroethylene in 4 strains of rats (ACI, August, Marshall, Osborne-Mendel. NTP TR 273, NIH publ. No. 88-2529 1987.

7. Herren-Freund, S.L., Pereira, M.A., Khoury, M.D., Olson, G. The carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid in mouse liver. Toxicol. Appl. Pharmacol. 90, 183-189 1987.

8. Bull, R.J., Sanchez, I.M., Nelson, M.A., Larson, J.L., and Lansing, A.J., Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate. Toxicology 63: 341-359 1990.

9. Deangelo, A.B., Daniel, F.B., McMillan, L., Wersing, P. and Savage, R.E.jr, Species and strain sensitivity to the induction of peroxisomal proliferation by chloroacetic acids. Toxicol. Appl. Pharmacol. 101,285-298 1989.

10. Daniel, F.B., Deangelo, A.B., Stober, J.A., Olson, G.R., Pages, N.P., Hepatocarcinogenicity of chloral hydrate, 2-chloroacetylaldehyde, and dichloroacetic acid in the male B6C3F1 mouse. Fundam. Appl. Toxicol. 19:159-168 1992.

11. US Environmental Protection Agency. Health assessment document for trichloroethylene, EPA/600/8-82/006F, Final Report 1985.

12. Butler, T.C. Metabolic fate of chloral hydrate J. Pharmacol. Exper. Ther. 97:84-92 1949.

13. Khimmerle, G., and Eden, A. Metabolism, excretion, and toxicology of trichloroethylene after inhalation. I. experimental exposure on rats. Arch. Toxikol. 30 115-126 1973a.

14. Cole, W.J., Mitchell, R.G., Salamonsen, R.F. Isolation, characterization and quantification of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography. J. Pharm. Pharmacol. 27:167 1975.

15. Bonse, G., Urban, T., Reichart, D., Henschler, D. chemical Reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. Biochem. Pharmacol. 24: 1829-1834 1975.

16. Byinton, K.H., and Leibman, K.C., Metabolism of

trichloroethylene in liver microsomes II Identification of the reaction product as chloral hydrate. Mol. Pharmacol. 1:147-154 1975.

17. Ikeda, M., Miyake, Y., Ogata, M. and Ohmori, S. Metabolism of trichloroethylene. Biochem. pharmacol. 29: 2983-2992 1980.

18. Miller, R.E., Guengerich, F.P. Oxidation of trichloroethylene by liver microsomal cytochrome p-450 : evidence for chlorine migration in the transition state not involving trichloroethylene oxide. Biochemistry 21:1090-1097 1980.

19. Sato, A., Nakajima, T., Fujiwara, Y. and Murayama, N. A pharmacokinetic model to study the excretion of trichloroethylene and its metabolites after an inhalation exposure Brit. J. Ind. Med 34: 56-63 1977.

20. Fernandez J.G., Droz, P.O. Humbert, B.E. and Caperos, J.R. Trichloroethylene exposure: Simulation of uptake, excretions, and metabolism using a mathematical model. Brit. J. Ind. Med. 34, 43-55 1977.

21. Ramsey, J.C., and Andersen, M.E. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol. Appl. Pharmacol. 73, 159-175 1984.

22. Andersen, M.E. , Gargas, M.L., Clewell, H.J., and Severyn, K.M. Quantitative evaluation of the metabolic interactions between trichloroethylene and 1, 1 dichloroethylene in vivo using gas uptake systems. Toxicol. Appl. Pharmacol. 89: 149-157 1987.

23. Fisher, J.W., Whittaker, T.A., Taylor, D.H., Clewell, H.J. and Andersen, M.E. Physiologically based pharmacokinetic modeling of the pregnant rat : A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 99: 395-414 1989.

24. Fisher, J.W., Whittaker, T.A., Taylor, D.H., Clewell, H.J. and Andersen, M.E. Physiologically based model of lactating rat and nursing pup : a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 102: 497-513 1990.

25. Dallas, C.E, Gallo, J.M., Ramanathan, R., Muralidhara, S. and Bruckner, J.V. Physiologically based pharmacokinetic modeling of inhaled Trichloroethylene in rats. Toxicol. and Appl. Pharmacol. 110: 303-314 1991.

26. Jepson, G.W., Hoover, D.K., Black, R.K., McCafferty, J.D., Mahle, D.A. and Gearhart, J.M. A partition coefficient method for non-volatile and intermediate volatility chemicals in biological tissues. Fund. Appl. Tox. 22:519-524 1994.